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TO ALL WHOM IT MAY CONCERN:

Be it known that WE, XIAO-ZHUO MICHELLE WANG, a citizen of The Peoples Republic of China, XAVIER GEORGES SARDA, a citizen of France, MICHAEL DAVID TOMALSKI, a citizen of the United States, and VINCENT PAUL MARY WINGATE, a citizen of the United States, whose post office addresses are 211 Tremont Circle, Chapel Hill, North Carolina, 27516; 6 Rue Daniel Fery, Sainte Genevieve Des Bois, France, 91700; 8116 North Creek Run, Raleigh, North Carolina, 27613; and 613 Churchill Drive, Chapel Hill, North Carolina, 27614, respectively, have invented an improvement in

HELIOTHIS GLUTAMATE RECEPTOR

of which the following is a

SPECIFICATION

**BACKGROUND OF THE INVENTION**

5           Glutamate-gated chloride channels are a family of ligand-gated chloride channels  
unique to invertebrates. Glutamate-gated chloride channels have been cloned from *Caenorhabditis*  
*elegans* (Cully et al. (1994) *Nature* 20:371; U.S. Patent No. 5,527,703), *Drosophila melanogaster*  
(Cully et al. (1996) *J. Biol. Chem.* 271:20187 and U.S. Patent No. 5,693,492), *Haemonchus*  
*contortus* (Delany et al. (1998) *Mol. Biochem. Parasit.* 97:177), *Lucilia cuprina* (GenBank Accession  
10 No. AAC31949) and *Schistocerca americana* (Cohen et al. (1999) 29th Annual Neuroscience  
Meeting, p. 199). The clones isolated from *C. elegans*, *D. melanogaster* and *S. americana* have been

5 functionally expressed in *Xenopus* oocytes, and shown to be activated by glutamate and avermectin. (Arena et al. (1991) *Molecular Pharm.* 40:368; Arena et al. (1992) *Molecular Brain Research* 15:339; U.S. Patent No. 5,693,492; U.S. Patent No. 5,527,703; Cohen et al., *supra*).

Because glutamate-gated chloride channels are specific to invertebrates, the channels provide a target for insecticides. In particular, the glutamate-gated chloride channels are the target  
10 of the avermectin class of insecticides. Avermectins are naturally occurring and synthetic macrocyclic lactones that are widely used in the treatment of parasites and insects.

Insects of the order lepidoptera are significant pests, and in particular the larvae are destructive defoliators. Further, lepidopteran pests are typically harder to control than diptera. Accordingly, there is a need to identify and develop safe and specific insecticides against  
15 lepidopteran pests. The present invention addresses this need by providing isolated nucleic acids encoding a lepidopteran glutamate-gated chloride channel, recombinant lepidopteran glutamate-gated chloride channels, and a method of identifying agents that modulate the activity of the channel.

## SUMMARY OF THE INVENTION

The present invention is directed to an isolated nucleic acid encoding a lepidopteran  
20 glutamate-gated chloride channel. In a preferred embodiment the nucleic acid is isolatable from *Heliothis virescens*. In another preferred embodiment the nucleic acid comprises a sequence encoding the amino acid sequence of SEQ ID NO. 14.

The present invention further comprises vectors comprising a nucleic acid encoding a lepidopteran glutamate-gated chloride channel, and host cells comprising the vectors.

5 Another aspect of the present invention provides a recombinant lepidopteran glutamate-gated chloride channel, and kits and compositions comprising a recombinant lepidopteran glutamate-gated chloride channel. A method for preparing a lepidopteran glutamate-gated chloride channel is also provided.

10 In yet another embodiment, the present invention provides a *Xenopus* oocyte comprising a nucleic acid encoding a lepidopteran glutamate-gated chloride channel, and a *Xenopus* oocyte expressing a functional lepidopteran glutamate-gated chloride channel.

The present invention further provides a method of identifying agents that modulate the activity of the lepidopteran glutamate-gated chloride channel, and agents identified by the method.

## 15 **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 presents electrophysiological recordings demonstrating the effect of glutamate on a lepidopteran glutamate-gated chloride channel expressed in oocytes.

## **DETAILED DESCRIPTION OF THE INVENTION**

20 The present invention is directed to isolated nucleic acids encoding lepidopteran glutamate-gated chloride channels. An isolated nucleic acid encoding a lepidopteran glutamate gated chloride channel is defined herein as a nucleic acid isolatable from an insect of the order lepidoptera and capable of encoding a functional glutamate-gated chloride channel. In a preferred embodiment, the nucleic acid is isolatable from *Heliothis*. In a more preferred embodiment, the nucleic acid is

5 isolatable from *Heliothis virescens*. A functional glutamate-gated chloride channel is defined herein as a protein having the ability to bind glutamate and thereby mediate chloride flux in a cell expressing the channel.

The isolated nucleic acid may be DNA or RNA, including cDNA and mRNA. In a preferred embodiment, the isolated nucleic acid has a sequence encoding the amino acid sequence  
10 of SEQ ID NO. 14. The ordinarily skilled artisan, with knowledge of the genetic code, can determine DNA and RNA sequences that encode the amino acid sequence set forth in SEQ ID NO. 14. Further, the sequence may be selected to optimize expression in a particular host organism by utilizing known preferred codons for the host organism of choice.

In another preferred embodiment, the isolated nucleic acid comprises the sequence  
15 set forth in SEQ ID NO. 13. In another preferred embodiment, the isolated nucleic acid comprises the sequence of nucleotides 144 through 1484 of SEQ ID No. 13. Fragments of a nucleic acid having the sequence of SEQ ID No. 13 that maintain the ability to encode a functional lepidopiteran glutamate-gated chloride channel are also encompassed by the present invention.

The present invention further encompasses nucleic acids isolatable from lepidoptera  
20 and capable of hybridizing under high stringency conditions to the complement of a nucleic acid having the sequence of nucleotides 144 through 1484 of SEQ ID NO: 13. Nucleic acid hybridization conditions are known to those of ordinary skill in the art and disclosed for example by Sambrook et al. (1989) *Molecular Cloning - A laboratory manual*, Cold Spring Harbor Laboratory Press. High stringency conditions are defined herein as 0.1 X standard saline citrate (SSC), 0.1% sodium dodecyl  
25 sulfate (SDS) at 60°C.

5 In another embodiment, the present invention provides isolated nucleic acids having at least about 80%, and preferably at least about 90%, and more preferably at least about 95% sequence identity to the nucleic acid having the sequence of nucleotides 144 through 1484 of SEQ ID NO: 13. Sequence identity is determined using the program Clustal W described by Higgins et al. (1994) Nucleic Acids Res. 22:4673 and may be calculated using the EMBL Nucleotide Sequence  
10 Database (<http://www.ebi.ac.uk/embl.html>). The ability of the isolated nucleic acid of the present invention to encode a functional glutamate-gated chloride channel can be determined by functional assays as described hereinbelow.

A protein having the amino acid sequence of SEQ ID NO: 14 has glutamate-gated chloride channel activity. Analysis of the amino acid sequence and alignment with the sequence of  
15 the *Drosophila* glutamate-gated chloride channel indicates that the sequence of SEQ ID NO: 14 contains four membrane spanning regions at amino acids 246-268, 274-293, 309-328 and 415-435. The amino terminal 20-30 amino acids encode a signal peptide. Amino acid changes may be tolerated in the signal peptide domain so long as the ability of the protein to insert into a selected cell membrane is maintained. Those of ordinary skill in the art can determine suitable modifications of  
20 the sequence of the signal peptide and can likewise determine the nucleic acid sequence encoding the modified signal peptide domain.

The nucleic acids of the present invention may be obtained by using a nucleic acid having the sequence of SEQ ID NO: 13 or a fragment thereof to probe a lepidopteran cDNA library. Such libraries may be made by well-known methods, described for example in Sambrook et al.,

5 supra, or may be obtained commercially. The identity of the nucleic acid may be confirmed by nucleotide sequencing or by expression and functional analysis as described hereinbelow.

The present invention is further directed to vectors comprising the isolated nucleic acids of the present invention. In the vectors of the present invention, the nucleic acid encoding a lepidopteran glutamate-gated chloride channel is operably linked to suitable transcriptional and/or  
10 translational regulatory elements to effect expression of the glutamate-gated chloride channel in a suitable host cell. The regulatory elements may be derived from mammalian, microbial, viral or insect genes, and include, for example, promoters, enhancers, transcription and translation initiation sequences, termination sequences, origins of replication, and sequences encoding leader and transport sequences. Suitable regulatory elements are selected for optimal expression in a desired  
15 host cell. Useful expression vectors can be constructed by methods known to one of ordinary skill in the art, and vectors into which the nucleic acid of the invention can be inserted are also commercially available. Recombinant viral vectors, including retrovirus, baculovirus, parvovirus and densovirus vectors are particularly preferred.

In a preferred embodiment the vector comprises a strong constitutive or inducible  
20 promoter operably linked to a nucleic acid encoding a lepidopteran glutamate-gated chloride channel. Suitable promoters are well known and readily available to one of ordinary skill in the art, and include for example, the polyhedrin promoter (Kitts et al., 1993, Bio Techniques, 14:810), heat shock promoter (Stellar et al., 1985, EMBO J., 4:167) and metallothionein promoter (Kaufman et al., 1989, Cell 59:359). Expression vectors can be constructed by well known molecular biological  
25 methods as described, for example, in Sambrook et al. (1989) Molecular Cloning: A Laboratory

5 Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., or any of a myriad of laboratory manuals on recombinant DNA technology that are widely available. Expression vectors into which the nucleic acids of the present invention can be cloned under the control of a suitable promoter are also commercially available.

10 Another embodiment of the present invention provides host cells containing the vectors described above. The host cell may be procaryotic or eukaryotic, including bacterial, yeast, insect or mammalian. Insect and mammalian cells are preferred. Particularly preferred host cells include insect cell lines, including for example *Spodoptera frugiperda* cells. The host cells may be transformed, transfected or infected with the expression vectors of the present invention by methods well-known to one of ordinary skill in the art. Transfection may be accomplished by known  
15 methods, such as liposome mediated transfection, calcium phosphate mediated transfection, microinjection and electroporation. Permanently transformed insect cell lines are particularly preferred. For example, insect cell lines such as *Drosophila* cell line SH1 can be transformed with the expression vectors of the present invention by commercially available lipofectin (GIBCO-BRL) to provide permanently transformed cell lines expressing a functional glutamate-gated chloride  
20 channel. In a preferred embodiment, the vector is designed such that expression of the protein is inducible.

Expression systems utilizing baculovirus vectors and insect host cells are preferred. The use of baculoviruses as recombinant expression vectors to infect lepidopteran insect cells is known in the art and described for example by Luckow et al. (1988) *Bio/Technology* 6:47-55 and  
25 Miller (1988) *Ann Rev Microbiol.* 42:177-199. The baculovirus vectors generally contain a strong

5 baculovirus promoter operably linked to a nucleic acid of the present invention such that the promoter directs expression of the lepidopteran glutamate-gated chloride channel. Baculovirus polyhedrin promoters such as the *Autographa californica* nuclear polyhydrosis virus polyhedrin promoter are preferred.

The baculovirus expression vectors of the present invention are made by inserting the  
 10 nucleic acid encoding the lepidopteran glutamate-gated chloride channel downstream of the polyhedrin promoter in a baculovirus transfer vector, for example pBacPac8 available from Clontech or Bac-to-Bac available from Life Technologies. Baculovirus transfer vectors further contain flanking baculovirus sequences that allow homologous recombination between the transfer vector and baculovirus DNA during co-transfection. The transfer vector containing the nucleic acid of the  
 15 invention and viral DNA are used to co-transfect insect cells. In a preferred embodiment the insect cells are *Spodoptera*. *Spodoptera frugiperda* cells including Sf9 are particularly contemplated. During co-transfection, homologous recombination results in the transfer of an expression cassette containing the polyhedrin promoter and the nucleic acid of the present invention to the polyhedrin locus of the viral DNA. The resulting recombinant virus is used to generate viral stocks by standard  
 20 methods. Insect host cells are infected with the recombinant virus to produce insect cells expressing the glutamate-gated chloride channel.

The present invention is further directed to recombinant glutamate-gated chloride channel. The recombinant lepidopterant glutamate gated chloride channel may be isolated in a membrane preparation or present in the cell membrane of the host cell in which it has been  
 25 recombinantly produced. Whole cells and membrane preparations comprising the recombinant



5 lepidopteran glutamate-gated chloride channel are particularly contemplated. Recombinant lepidopteran glutamate-gated chloride channel is useful, for example, to screen potential insecticides by specific binding assays or functional assays.

The present invention further provides a method of making a recombinant lepidopteran glutamate-gated chloride channel. Recombinant lepidopteran glutamate-gated chloride  
10 channel is made by transforming, transfecting or infecting a suitable host cell with an expression vector comprising a nucleic acid encoding a lepidopteran glutamate-gated chloride channel, culturing the host cell under conditions suitable for expression, and optionally recovering the recombinant lepidopteran glutamate-gated chloride channel. A suitable host cell is any cell in which the nucleic acid encoding the transporter can be expressed to provide a functional glutamate-gated chloride  
15 channel. In a preferred embodiment, the recombinant lepidopteran glutamate-gated chloride channel is made in insect cells, preferably *Spodoptera frugiperda* 9 (Sf9), by infecting the insect cells with a recombinant virus in which the nucleic acid of the invention is under the control of a promoter suitable for use in Sf9 cells, such as a polyhedrin or TE1 promoter, and culturing the cells under conditions suitable for expression of the recombinant lepidopteran glutamate-gated chloride channel.  
20 In another preferred embodiment, the recombinant lepidopteran glutamate-gated chloride channel is made in permanently transformed cell lines as described above.

A functional lepidopteran glutamate-gated chloride channel can be identified by one of ordinary skill in the art by functional assays. An exhaustive review of techniques and protocols is provided in Rudy et al., eds. (1992) *Methods in Enzymology* 207, Academic Press, Inc., San  
25 Diego, CA. For example, two-electrode voltage clamp recordings of host cells or oocytes expressing

5 the lepidopteran glutamate-gated chloride channel can be used to assess chloride flux in response to application of glutamate or ivermectin phosphate. Dose-dependent glutamate-evoked currents indicate the presence of a functional glutamate-gated chloride channel. Also, the activation of a membrane current by about 100  $\mu$ M glutamate or 1  $\mu$ M ivermectin phosphate is indicative of a functional glutamate-gated chloride channel.

10 The present invention further provides an amphibian oocyte comprising a nucleic acid encoding a functional glutamate-gated chloride channel, and an amphibian oocyte expressing a functional glutamate-gated chloride channel. The oocytes are useful as a system for screening potential insecticides useful against insects of the order lepidoptera. Such oocytes can be made using the nucleic acids of the invention and methods known in the art. In a preferred embodiment, the  
15 oocyte is *Xenopus laevis* oocyte. For example, expression vectors containing cDNA encoding the glutamate-gated chloride channel under the control of a strong promoter can be injected into the nuclei of oocytes, after which oocytes are incubated for from one to several days, followed by assessment for the presence of functional glutamate-gated chloride channel. Alternatively, mRNA can be synthesized in vitro from cDNA encoding the glutamate-gated chloride channel, and injected  
20 into oocytes, followed by assessment for the presence of functional glutamate-gated chloride channels as described hereinabove.

The present invention further provides methods of identifying agents that modulate the activity of a lepidopteran glutamate-gated chloride channel, and also encompasses novel agents identified by such methods. The agent may be an agonist, i.e. it mimics the action of glutamate by  
25 activating chloride flux, or an antagonist, i.e. it decreases the glutamate-activated chloride flux. The

5 agent may be nucleic acid, peptide, protein, a non-protein organic molecule, or any other molecule capable of modulating the activity of the glutamate-gated chloride channel.

A method of identifying an agonist comprises applying the putative agonist to a Xenopus oocyte, a cell or membrane expressing the lepidopteran glutamate-gated chloride in the presence of chloride ions, and measuring chloride flux, wherein flux of chloride is indicative of an  
10 agonist. A method of identifying an antagonist comprises applying glutamate to Xenopus oocyte or a cell or membrane expressing the lepidopteran glutamate-gated chloride channel in the presence of chloride ions and measuring chloride flux; applying the putative antagonist and then, for example after about one minute, applying glutamate to the cell or membrane and measuring chloride flux; and comparing the chloride flux obtained in the presence of glutamate alone to the flux obtained under  
15 similar conditions in the presence of both putative antagonist and glutamate, wherein a decrease in flux of chloride observed in the presence of the putative antagonist relative to the flux observed in the presence of glutamate alone is indicative of an antagonist. In a preferred embodiment, chloride flux is measured by voltage clamp electrophysiology. In another preferred embodiment, the cell is an recombinant baculovirus-infected Sf9 cell or a permanently transformed cell line. In another  
20 preferred embodiment, the concentrations of agonists, antagonists and GABA are from about 0.1 nM to about 1.0 mM.

Agonists and antagonists against the lepidopteran glutamate-gated chloride channel can also be identified by ligand binding assays. Agonists and antagonists are identified by their ability to displace radiolabeled ligands known to act as agonists or antagonists, respectively. The  
25 recombinant glutamate-gated chloride channel, present in an oocyte, cell, or membrane, (preferably

5 a membrane) is incubated with radiolabeled ligand and unlabeled candidate agonist or antagonist. After incubation, the incubation mixture is filtered, and radioactivity retained on the filters is measured by methods known in the art, for example liquid scintillation counting. The ability of the candidate compound to inhibit specific binding of the radiolabeled ligand provides a measure of the compound's agonist or antagonist activity. Suitable ligands include glutamate and ivermectin  
10 phosphate.

Agents identified by the foregoing methods may be useful as insecticides. Agents identified by the present methods may be assessed for insecticidal activity by in vitro and in vivo methods known in the art.

Another embodiment of the present invention provides a composition comprising a  
15 recombinant lepidopteran glutamate-gated chloride channel in a cell membrane. The composition may be a membrane preparation, including a freeze dried membrane preparation, or an intact cell or oocyte expressing the functional lepidopteran glutamate-gated chloride channel. The composition is useful, for example, to screen for potential insecticides by functional or specific binding assays. The composition may further comprise appropriate carriers or diluents, including, for example,  
20 physiological buffers.

The present invention further provides a kit for identifying agents that modulate the activity of a lepidopteran glutamate-gated chloride channel. The kit contains a first container containing a recombinant lepidopteran glutamate-gated chloride channel in a cell membrane. The membrane may be in the form of a membrane preparation, including a freeze dried membrane  
25 preparation, or an insect cell or oocyte expressing the functional lepidopteran glutamate-gated

5 chloride channel. The kit of the present invention optionally further comprises glutamate. The compositions and kits of the present invention are useful for identifying insecticides.

All references cited herein are incorporated in their entirety.

The following nonlimiting examples serve to further illustrate the present invention.

### EXAMPLE 1

#### 10 RNA Isolation

*Heliothis virescens* embryo were isolated from eggs just before hatching obtained from Rhone-Poulenc in-house insectary, and *Heliothis virescens* muscles were obtained by dissecting early 5<sup>th</sup> instar *Heliothis virescens* larva to remove the fat body, gut, and central nervous system. Eggs and remaining larva skins were frozen in liquid nitrogen, and ground to powder. Powders were added  
15 to lysis buffer, and homogenized before proceeding with manufacturer's instruction for total RNA isolation using Poly(A) Pure™ kit from Ambion. Poly A<sup>+</sup> RNA were selected twice by going through a oligo dT column. The RNA recovered from the column was dissolved in diethylpyrocarbonate (DEPC)-treated water. RNA was quantified by spectrophotometry and separated on a denaturing agarose gel to check its integrity before use in RT-PCR and cDNA library  
20 construction.

#### PCR Using Degenerate Primers:

Two degenerate oligonucleotides, mw 01 and mw 02, were designed and synthesized from highly conserved regions found in glutamate-gated chloride channel family following the amino acid

sequence for *Drosophila melanogaster* GluCl (Cully et al. (1996) J. Biol. Chem. 271:20187), *Caenorhabditis elegans* GluCl- $\alpha$  and *C. elegans* GluCl- $\beta$  (Cully et al. (1994) Nature 20:371). Primer mw 01 has the sequence 5'-GGATGCC(ATGC)GA(TC)(TC)T(ATGC)TT(TC)TT-3'. (SEQ ID NO.: 1) Primer mw02 has the sequence 5'-TC(ATGC)A(AG)CCA(AG)AA(ATGC)(GC)(AT)(ATGC)ACCC-3'. (SEQ ID NO: 2). The primer mw 01 was located upstream of the transmembrane (TM) domain 1, while downstream primer mw 02 was located within the TM domain 1. The primer mw 02 was used to synthesize first strand cDNA from mRNA isolated from *Heliothis* embryo using Boehringer Mannheim's 1<sup>st</sup> Strand cDNA Synthesis Kit for RT-PCR. The cDNA was used as the template for a hot start PCR mix (100  $\mu$ l) containing: 0.8 mM dNTP's, 2 mM MgCl<sub>2</sub>, 1.2 pmol / $\mu$ l degenerate primers and 5 U *Pfu* DNA polymerase (Stratagene). The amplification was performed using 35 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min and elongation at 72°C for 1 min. The denaturation step of the first cycle was 5 min long and the elongation step of the last cycle was 10 min (Perkin Elmer, DNA Thermal Cycler 480) (Sambrook et al., 1989, Molecular Cloning – A laboratory Manual. Cold Spring Harbor Laboratory Press).

PCR generated a 451 base pair (bp) fragment which was cloned into the pCR-Blunt vector (Invitrogen) to produce pE6 and sequenced. The amplified fragment had the following sequence:  
 5'-GGA TGC CGG ATT TGT TTT TCT CCA ACG AGA AGG AAG GTC ATT TCC ACA AC  
 A TCA TCA TGC CGA ACG TGT ACA TCC GGA TCT TCC CCA ACG GCA ACG TGC T GT  
 ACA GCA TCC GAA TCT CCT TGA CGC TCT CGT GCC CCA TGA ACC TCA AGT TGT  
 ACC CCC TGG ATA AGC AGA CCT GCT CGC TCA GGA TGG CTA GTT ATG GT T GGA

CCA CAG ACG ACT TAG TGT TCC TAT GGA AGG AAG GCG ACC CGG TGC AGG TGG  
TGA AGA ACT TAC ACC TGC CTC GGT TCA CGC TGG AGA AGT TCC TCA CTG ACT  
ACT GCA ACA GTA AGA CTA ATA CCG GTG AAT ACA GTT GCC TGA AGG TAG ACT  
TGC TCT TCA AAC GCG AGT TCA GTT ACT ACC TGA TCC AGA TCT ACA TTC CGT GCT  
GCA TGC TGG TCA TCG TGT CCT GGG TCA CCT TTT GGC TCG A-3' (SEQ ID NO: 3).

#### Rapid Amplification of cDNA Ends (RACE-PCR):

RACE reactions (Frohman et al. 1988 Proc. Natl. Acad. Sci. USA 85:8998), used to obtain the 5' and 3' ends of the *Heliothis virescens* mRNA, was performed using synthesized double stranded cDNA as the template. Two microgram of polyA mRNA from either *Heliothis virescens* embryo or muscle were used to synthesize cDNA with a Marathon cDNA amplification kit (CLONTECH) following the manufacturer's instructions. Specific primer mw03, derived from 451 bp fragment and having the sequence 5'-CCTGCACCGGGTCGCCTTCCTTCC-3', (SEQ ID NO:4) along with the adaptor primer (AP1, 5'-CCATCCTAATACGACTCACTATAGGGC-3') (SEQ ID NO: 5) provided in the kit were used for the amplification of 5' cDNA end. Primer mw04, derived from 451 bp fragment and having the sequence 5'-TACAGCATCCGAA TCTCCTTGACGC, (SEQ ID NO: 6) along with the primer AP1 were used for the amplification of 3' cDNA end. The PCR reactions were carried under the same conditions as in above section except using "touchdown PCR", which was performed using 5 cycles of denaturation at 94°C for 30 sec, annealing and elongation at 72°C for 4 min; 5 cycles of denaturation at 94°C for 30 sec, annealing and elongation at 70°C for 4 min; and 25 cycles of denaturation at 94°C for 20 sec, annealing and elongation at 68°C

5 for 4 min. The denaturation step of the first cycle was 1 min at 94°C. One tenth of the PCR reaction (10 µl) was separated on a 1.2% agarose gel containing 1 µg/ml ethidium bromide. The amplified fragments from both 5'RACE and 3'RACE were cloned into pCR2.1 vector (Invitrogen) to produce plasmids designated p5'E4 and p3'M5, respectively. Fragment in p5'E4 has the following sequence:

CGC TGA GCA TTG CGA ACT ACG CCT TCA ACA TTG TTT TTT TAA ACA AGC ACC

10 GTT TTT TAA TTT TAA AAG CTC TCA TTA AAG GTT TTA TTT GAA GGA AAG TTG TGA

CAG CAA CCG GAG TCG TTT AGA ATG GGA CTT TGT TGA GTC AGA GGA TGG ACA

TCC CGC GGC CAT CAT GCG CCC TCG TAT TGG TGT TGT TAT TTG TCA CCC ATC TCT

CAG AAT GCA TGA ACG GTG GGA AGA TCA ACT TCC GAG AGA AGG AGA AGC AGA

TCC TGG ATC AGA TCC TGG GCC CCG GGA GGT ACG ACG CCA GGA TCA GAC CCT

15 CGG GGA TCA ACG GCA CCG ATG GGC CAG CGG TAG TGA GCG TCA ATA TAT TTG

TCC GAA GTA TAT CAA AGA TCG ATG ATG TCA CAA TGG AAT ACT CCG TAC AGT

TAA CGT TTC GGG AAC AAT GGT TAG ATG AAC GGC TCA AAT TCA ATA ATC TTG

GAG GTC GCC TCA AAT ACC TGA CGC TTA CCG AAG CCA ACA GAG TCT GGA TGC

CTG ATC TAT TCT TCT CCA ACG AGA AGG AAG GTC ATT TCC ACA ACA TCA TCA

20 TGC CGA ACG TGT ACA TCC GAA TCT TCC CCA ACG GCA ACG TGC TGT ACA GCA

TCC GAA TCT CCC TGA CGC TCT CGT GCC CCA TGA ACC TCA AGT TGT ACC CCC

TGG ATA AGC AGA CCT GCT CGC TCA GGA TGG CTA GTT ATG GTT GGA CCA CAG

ACG ACT TAG TGT TCC TAT GGA AGG AAG GCG ACC CGG TGC AGG. (SEQ ID NO: 7)

Fragment in p3'M5 has the following sequence: 5'-CGC TCT CGT GCC CCA TGA ACC

25 TCA AGT TGT ACC CCC TGG ATA AGC AGA CCT GCT CGC TCA GGA TGG CTA GTT



5 ATG GTT GGA CCA CAG ACG ACT TAG TGT TCC TAT GGA AGG AAG GCG ACC CGG  
TGC AGG TGG TGA AAA ACT TAC ACC TGC CTC GGT TCA CGC TGG AGA AGT TCC  
TCA CTG ACT ACT GCA ACA GTA AGA CTA ATA CCG GTG AAT ACA GTT GCC TGA  
AGG TAG ACC TGC TCT TCA AAC GCG AGC TCA GTT ACT ACC TGA TCC AGA TCT  
ACA TTC CGT GCT GCA TGC TGG TCA TCG TGT CCT GGG TGT CCT TCT GGC TGG ACC  
10 AGG GAG CTG TGC CTG CGA GGG TCT CAC TAG GAG TGA CGA CTT TAC TTA CAA  
TGG CGA CCC AGT CGT CAG GCA TCA ACG CGT CCC TAC CAC CGG TGT CCT ACA  
CGA AAG CCA TTG ATG TCT GGA CTG GTG TAT GTC TCA CAT TCG TAT TCG GAG  
CGC TAC TAG AGT TCG CGC TCG TCA ACT ATG CGT CT C GCT CTG ACA TGC ACC  
GAG AGA ACA TGA AGA AAG CGA GAC GGG AGA TGG AA G CAG CCA GCA TGG ATG  
15 CTG CCT CAG ATC TCC TTG ATA CAG ATA GCA ACA CC A CC TTT GCT ATG AAA CCC  
TTG GTG CGC GGC GGC GTG GTG GAA TCC AAG ATG CGG CAG TGC GAG ATC CAC  
ATC ACC CCG CCG CGG AAG AAC TGC TGC CGC CTG TGG ATG TCC AAG TTC CCC  
ACG CGC TCC AAG AGG ATAGAC GTCATC TCC AGG ATC ACC TTC CCA CTT GTG TTC  
GCT CTG TTT AAC CTG GCT TAC TGA ATG AAG CAG AGA AAC TCC TCC TTT GCG  
20 CAC AGA AAT CCT GAA GAG ACT GAA CAA CGA AGT TTC CTA ACC ACA ATC ATT  
GCT ATG ATT ATA CCG AGA ATT TAT TTT ATA CTA ATT GTT GTG ACC ACA CGG TTT  
TAA\_CGT AGC TTG GAT CCA CGC GGT GTT AAT ATT TGT TGA TCG CTT AGA ATA  
AAT AAA TAT GCT TTG TTG AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA-  
3' (SEQ ID NO: 8).

## 5 Generation of Full-Length cDNA by PCR:

5' and 3'-end gene specific primers (GSP) were designed based on the sequence obtained from previous 5'- and 3'-RACE products. 5' GSP1 has the following sequences: 5'-GCTGAGCATTGCGAACTACGCCTTCAAC 3', (SEQ ID NO: 9) and 3' GSP2 has the following sequences: 5'-TAACACCGCGTGGATCCAAGCTACG 3' (SEQ ID NO: 10). Full-length cDNAs from both *Heliothis* embryo and muscle were generated using 5' GSP1 and 3' GSP2 in a long distance PCR reaction which used following cycle condition: 1 cycle of denaturation at 94°C for 1 min, and 25 cycles of denaturation at 94°C for 30sec and annealing and extension at 72°C for 5 min using *pfu* as polymerase. The amplified fragments from both *Heliothis* embryo and muscle were cloned into pCR2.1-TOPO vector (Invitrogen) to generate plasmids HEG3E(4)-2 and HEGM(1)-3. HEG3E(4)-2 has the following sequences (SEQ ID NO: 11):

1 CTGAGCATTG CGAACTACGC CTTCAACATT GTTCTTTAA ACAACACCG TTTTTTAATT  
61 TTAATAGCAC TCATTAAAGG TTTTATTTGA AGGAAAGTTG TGACAGCAAC CGGAGTCGTT  
121 TAGAATGGGA CTTTGTGAG TCGGAGGATG GACATCCCGC GGCCATCATG CGCCCTCGTA  
181 TTGGTGTGT TATTGTCTCA CCATCTCTCA GAATGCATGA ACGGTGGGAA GATCAACTTT  
20 241 CGAGAGAAGG AGAAGCAGAT CCTGGATCAG ATCCTGGGCC CCGGGAGGTA CGACGCCAGG  
301 ATCAGACCCCT CGGGGATCAA CGGCACTGAT GGGCCAGCGG TAGTGAGCGT CAATATATTT  
361 GTCCGAAGTA TATCAAAGAT CGATGACGTC ACAATGGAAT ACTCCGTACA ATTAACGTTT  
421 CGGAACAAT GGTTAGATGA ACGGCTCAA TTCAATAATC TTGGAGTGC CCTCAAATAC  
481 CTGACTGTA CTGAAGCCAA CAGAGTCTGG ATGCCTGATC TATTCTTCTC CAACGAGAAG  
25 541 GAAGGTCATT TCCACAACAT CATCATGCCG AACGTGTACA TCCGAATCTT CCCCAACGGC  
601 AACGTGCTGT ACAGCATCCG AATCTCCCTG ACGCTCTCGT GCCCATGAA CCTCAAGTTG  
661 TACCCCTGG ATAAGCAGAC CTGCTCGCTC AGGATGGCTA GTTATGGTTG GACCACAGAC  
721 GACTTAGTGT TCCTATGGAA GGAAGGCGAC CCGGTGCAGG TGGTGAAAAA CTTACACCTG  
781 CCTCGGTTCA CGCTGGAGAA GTTCCTCACT GACTACTGCA ACAGTAAGAC TAATACCGGT

5 841 GAATACAGTT GCCTGAAGGT AGACCTGCTC TTCAAACGCG AGTTCAGTTA CTACCTGATC  
 901 CAGATCTACA TTCCGTGCTG CATGCTGGTC ATCGTGTCTT GGGTGTCTT CTGGCTGGAC  
 961 CAGGGAGCTG TGCCTGCGAG GGTCTCACTA GGAGTGACGA CTTTACTTAC AATGGCGACC  
 1021 CAGTCGTCAG GCATCAACGC GTCCCTACCA COGGTGTCTT ACACGAAAGC CATTGATGTC  
 1081 TGGACTGGGT TATGTCTCAC ATTCTGATTC GGAGCGCTAC TAGAGTTTGC GCTCGTCAAC  
 10 1141 TATGCGTCTC GCTCTGACAT GCACCGAGAG AACATGAAGA AAGCGAGACG GGAGATGGAA  
 1201 GCAGCCAGCA TGGATGCTGC CTCAGATCTC CTTGATACAG ATAGCAACAC CACCTTTGCT  
 1261 ATGAAACCTT TGGTGCCTGG CGGCGTGGTG GAATCCAAGA TGCGGCAGTG CGAGATCCAC  
 1321 ATCACCCCGC CGCGAAGAA CTGCTGCCGC CTGTGGATGT CCAAGTTCCC CACGCGCTCC  
 1381 AAGAGGATAG ACGTCATCTC CAGGATCACC TTCCCACTTG TGTTCTGCTCT GTTTAACCTG  
 15 1441 GCTTACTGAA TGAAGCAGAG AAATCTCTCC TTTGCGCACA GAAATCCTGA AGAGACTGAA  
 1501 CAACGAAGTT TCCTAACCAC AATCATTTGCT ATGATTATAC CGAGAATTTA TTTTATACTA  
 1561 ATTGTTGTGA CCACACGGTT TTAACGTAGC TTGGATCCAC GCGGTGTTA

HEGM(1)-3 has the following sequence (SEQ ID NO: 12):

1 AGGTGCGGAC GTCTGCACTT GCGAATCGAA GTGATAGAAA ATAGTTGAT GAATACGGGA  
 20 61 GTTTGAGTGG AGTGATTTAT AATTCGGAGG ATGGACATCC CGCGGCCATC ATGCGCCCTC  
 121 GTATTGGTGT TGTTATTTGT CACCCATCTC TCAGAATGCA TGAACGGTGG GAAGATCAAC  
 181 TTTGAGAGA AGGAGAAGCA GATCCTGGAT CAGATCCTGG GCCCGGGAG GTACGACGCC  
 241 AGGATCAGAC CCTCGGGGAT CAACGGCACT GGCTATGCGC CAACGTTAGT CCATGTCAAC  
 301 ATGTATCTAC GGTCCATCAG CAAAATAGAT GATTACAAA TGAATACTC CGTACAATTA  
 25 361 ACGTTTCGGG AACAAATGGTT AGATGAACGG CTCAAATTCA ATAATCTTGG AGGTGCGCTC  
 421 AAATACCTGA CACTGACTGA AGCCAACAGA GTCTGGATGC CTGATCTATT CTTCTCCAAC  
 481 GAGAAGGAAG GTCATTTCCA CAACATCATC ATGCCGAACG TGTACATCCG GATCTTCCCC  
 541 AACGGCAACG TGCTGTACAG CATCCGAATC TCCCTGACGC TCTCGTGCCC CATGAACCTC  
 601 AAGTTGTACC CCCTGGATAA GCAGACCTGC TCGCTCAGGA TGGCTAGTTA TGGTTGGACC  
 30 661 ACAGACGACT TAGTGTTCCT ATGGAAGGAA GCGGACCCGG TGCAGGTGGT GAAAAACTTA  
 721 CACCTGCCTC GGTTACGCTT GGAGAAGTTC CTCCTGACT ACTGCAACAG TAAGACTAAT

5           781 ACCGGTGAAT ACAGTTGCCT GAAGGTAGAC CTGCTCTTCA AACGCGAGTT CAGTTACTAC  
             841 CTGATCCAGA TCTACATTC GTGCTGCATG CTGGTCATCG TGCTCTGGGT GTCCTTCTGG  
             901 CTGGACCAGG GAGCTGTGCC TGCAGGGGTC TCACTAGGAG TGACGACTTT ACTTACAATG  
             961 GCGACCCAGT CGTCAGGCAT CAACGCGTCC CTACCACCGG TGTCTACAC GAAAGCCATT  
             1021 GATGTCTGGA CTGGGTTATG TCTCACATTC GTATTGGGAG CGCTACTAGA GTTTGCGCTC  
 10           1081 GTCAACTATG CGTCTCGCTC TGACATGCAC CGAGAGAACA TGAAGAAAGC GAGACGGGAG  
             1141 ATGGAAGCAG CCAGCATGGA TGCTGCCTCA GATCTCCTTG ATACAGATAG CAACACCACC  
             1201 TTTGCTATGA AACCCTTGGT GCGCGGCGGC GTGGTGGAAT CCAAGATGCG GCAGTGCGAG  
             1261 ATCCACATCA CCCC GCCGCG GAAGAACTGC TGCCGCTGT GGATGTCCAA GTTCCCCACG  
             1321 CGCTCCAAGA GGATAGACGT CATCTCCAGG ATCACCTTCC CACTTGTGTT CGCTCTGTTT  
 15           1381 AACCTGGCTT ACTGTTGGGG GGGCAAGAGG GGGGCGGTGG CTGCTACCAT GTCTTGCAGG  
             1441 AGCGATGAGA CTATTAATGC TATTTATAAG CTGATACAGA ATGAAGCAGA GAAACTCCTC  
             1501 CTTTGCGCAC AGAAATCCTG AAGAGACTGA ACAACGAAGT TTCCTAACCA CAATCATTGC  
             1561 TATGATTATA CCGAGAATTT ATTTTATACT AATTGTTGTG ACCACACGGT TTAAAGCTAG  
             1621 CTTGGATCCA CGCGGTGTTA

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20

**EXAMPLE 2**

Isolation of full-length clone by screening of cDNA library:

Compared to the glutamate-gated chloride channel clones from *Drosophila* and *C. elegans*, clone HEG3(E)-2 has a stop codon within the M4 transmembrane domain, whereas clone HEGM(1)-3 has an unusual long 3' sequence after the M4 transmembrane domain. It is unclear whether these  
 25 two clones resulted from different RNA splicing or due to the errors introduced by PCR polymerase during the RACE reaction. cDNA libraries of *Heliothis virescens* embryo and muscle were constructed using 7.5 µg of each of isolated polyA mRNA with Stratagene's cDNA Synthesis kit. The cDNAs were made according to the manufacturer's instructions and then cloned into the lambda

5 ZAP expression cloning vector and packaged with Gigapack III Gold packaging system (Stratagene) following the manufacturer's instructions. Thus two non-amplified libraries of  $5 \times 10^5$  recombinants were made and then amplified.

Clone HEG3(E)-2 insert was cut out from its vector by SacI enzyme, and was labeled with  $^{32}\text{P}$  using Boehringer Mannheim's Random Primed DNA Labeling Kit (Ca # 1004760). Part of the amplified *Heliothis virescens* embryo library was plated out on 10 large 150-mm NZY agar plate at 50,000 pfu/plate. Phage particles were transferred to nitrocellulose membranes. Membranes were denatured in a 1.5 M NaCl and 0.5 M NaOH denaturation solution for 5 minutes, neutralized in a 1.5 M NaCl and 0.5 M Tris-Cl (pH 8.0) neutralization solution for 5 minutes and rinsed in a 0.2 Tris-Cl (pH 7.5) and 2 x SSC buffer for 2 minute. DNA was crosslinked to the membranes using the Stratalinker UV crosslinker (CL-100 Ultraviolet Crosslinker, UVP). Prehybridization was performed in a 50 ml solutions containing: 25 ml of formamide, 12.5 ml of 20 x SSC, 0.5 ml of 10% SDS and 5 ml of Derhardt solution at 42C for 3 –4 hours. Labeled probes were added to the prehybridization solution at  $1.84 \times 10^5$  dpm/ml  $^{32}\text{P}$  and hybridization was continued at 42°C for 24 hours. Membranes were washed twice for 15 minutes in low stringency conditions (2 x SSC/0.1%SDS, room temperature), twice for 15 minutes in high stringency conditions (0.2 x SSC/0.1%SDS, 42C), and once for 15 minutes in higher stringency conditions (0.1 x SSC/0.1%SDS, 42C). Ten positive clones were identified and plaques were purified, and secondary and tertiary screenings were performed using the same primer with positive clones to make sure that each positive plaque was very well separated. The phagemids containing the inserts were excised following the manufacturer's instruction (Stratagene). Two clones which have the same full-length

5 sequences of glutamate-gated chloride channels, were designated HEGE2. The following DNA  
sequence (SEQ ID NO: 13) for clone HEGE2 was determined:

1 ACCAGGCGAA CTACGCCTTC AACATTGTTT TTTTAAACAA ACACCGTTTT TTAATTTTAA  
61 TAGCTCTCAT TAAAGGTTTT ATTTGAAGGA AAGTTGTGAC AGCAACCGGA GTCGTTTAGA  
121 ATGGGACTTT GTTGAGTCGG AGGATGGACA TCCC GCGGCC ATCATGCGCC CTCGTATTGG  
10 181 TGTGTATTATT TGTCACCCAT CTCTCAGAAT GCATGAACGG TGGGAAGATC AACTTTCGAG  
241 AGAAGGAGAA GCAGATCCTG GATCAGATCC TGGGCCCGCG GAGGTACGAC GCCAGGATCA  
301 GACCCTCGGG GATCAACGGC ACTGATGGGC CAGCGGTAGT GAGCGTCAAT ATATTTGTCC  
361 GAAGTATATC AAAGATCGAT GACGTCACAA TGAATACTC CGTACAGTTA ACGTTTCGGG  
421 AACAAATGGT AGATGAACGG CTCAAATTCA ATAATCTTGG AGGTCGCCTC AAATACCTGA  
15 481 CACTGACCGA AGCCAACAGA GTCTGGATGC CTGATCTATT CTTCTCCAAC GAGAAGGAAG  
541 GTCATTTCCA CAACATCATC ATGCCGAACG TGTACATCCG GATCTTCCCC AACGGCAACG  
601 TGCTGTACAG CATCCGAATC TCCTTGACGC TCTCGTGCCC CATGAACCTC AAGTTGTACC  
661 CCCTGGATAA GCAGACCTGC TCGCTCAGGA TGGCTAGTTA TGGTTGGACC ACAGACGACT  
721 TAGTGTTCCT ATGGAAGGAA GGCGACCCGG TGCAGGTGGT GAANAACCTA CACCTGCCTC  
20 781 GGTTCACGCT GGAGAAGTTC CTCCTGACT ACTGCAACAG TAAGACTAAT ACCGGTGAAT  
841 ACAGTTGCCT GAAGGTAGAC TTGCTCTTCA AACGCGAGTT CAGTTACTAC CTGATCCAGA  
901 TCTACATTCC GTGCTGCATG CTGGTCATCG TGTCTTGGGT GTCCTTCTGG CTGGACCAGG  
961 GAGCTGTGCC TGCAGGGGTC TCACTAGGAG TGACGACTTT ACTTACAATG GCGACCCAGT  
1021 CGTCAGGCAT CAACGCGTCC CTACCACCGG TGTCTTACAC GAAAGCCATT GACGTCTGGA  
25 1081 CTGGTGTATG TCTCACATTC GTATTCGGAG CGCTACTAGA GTTCGCGCTC GTCAACTATG  
1141 CGTCTCGCTC TGACATGCAC CGAGAGAACA TGAAGAAAGC GAGACGGGAG ATGGAAGCAG  
1201 CCAGCATGGA TGCTGCCTCA GATCTCCTAG ACACAGATAG CAACACCACC TTTGCTATGA  
1261 AACCCTTGGT GCGCGGCGGC GTGGTGAAT CCAAGATGCG GCAGTGCGAG ATCCACATCA  
1321 CCCC GCGCG GAAGAACTGC TGCCGCTGT GGATGTCCAA GTTCCCCACG CGCTCCAAGA  
30 1381 GGATAGACGT CATCTCCAGG ATCACCTTCC CACTTGTGTT CGCTCTGTTT AACCTGGCTT  
1441 ACTGGTCGAC GTACCTGTTT CCGCAGCAGG ACGAGGAGAA GTGATTCTCC GAGTCCCTGG  
1501 AGAGGGGCGT GGGGCGCGC GTGCAGCTGG TGGCGGCGT CGTGATGCCC TACGTGCTGT  
1561 TCGTGGTGGC GTACTCGCTG TGCTTCCGG CCGCGGCCCC GCCCCTTCG CCCCCGCGG  
1621 CGCCCGCGCC CGCGCCCGCG CCCGCACCCT CCCGCCGAG CGCGCGCGCA CGCACACAAG

5 1681 CACACCCACC TAGCCCGCTC TAGCGAACTC ACCCCATTCA TTATCGTGAC ATATTATATT  
1741 ATCGTGTATT TTAATCGACG TCTTCCTCGT GGCAGCGTTA TTCCCACTCA GTATTGATG  
1801 GCGTTAGTGT AATTAGTAAA GCTCAAGTGT CTATTTGTAT ATATATGTGA CCCCCTGCC  
1861 AGTTTAGACC AAGCCTCCGT TTTTAAATTG AAGCAGTTG AGAAAAACGG TAAAAATAGA  
1921 CTCAATTTTG ATTGGTCATC TAAACAGCAG AACTTTTATT CGGCACTTAT AAAGTCCTCA  
10 1981 ATTATTTGTG TACAAAAATA AATATTTTAC TTTCGAGAA TTAAAAATTT TCGATAATTT  
2041 TACCAATGAT ATGACTCCTT GTATGGATTG GTATGTAATG TAAACCTAGG TTAAGATATA  
2101 AGAGGAATCC CAGAGGTCC CGCATATTAC TTAGCCTTT AAAGTAAGGT AAATAAGGAC  
2161 TAGAATGGCA CTAATGTGTA GTGGAAGTGG GGTATTATTT AGTAGTTTC ACTCTACAGT  
2221 ACGTGAAGT GACTAGATCT ACTAGCAAAT AGAGTTGATC AATTTTCATG TCGAAATGTT  
15 2281 CACAGATATT GTATAAACCG CTGGAGGTAA ACAGCTATCA ACAATGTAAC ACCAAATACC  
2341 ATCAGAATCA AGCAAAACCA TGGAAATTTT GCTAATCGAA AAGTTGTAAC TGTATTATCTA  
2401 TGGCAGGTAT AATTGGCCTA GTAATGTATC GTGTAGTATC ATTTACAACA CATATTAECT  
2461 ATTAACCACA TTATGTGAAA GAAGGAATTT ATAAAAAAA CCTATTAAA TATATATTAG  
2521 ATAAGTATTA TTAATTGGAT ATTCTCTGTC TGGGGATTTT AATATGAATC TTACCTTTAA  
20 2581 ATAAGTTTGA TCTCACTAGA CGTTGCAAAT GGATACCCCA AATACCTTTT CCGCATTAAA  
2641 AGGTATTATT TTAACAAATG TATCTTCCC CGTCAATGTT TTAAGACTAC GTATCTACAT  
2701 AAAATGATGT ATTGTTTATA CAATACTATT TCAAAATGCA AGAACAACGT AAAGTGCATT  
2761 TCATTGATGT TTGTGTATGT AGATGACATT AGTATTTTAC CCAAAAATAC TGATATTAAA  
2821 ATTCCCAGTA AGATTCTAGT GTAAATGGTA AACGTGTAAA TAGTTGGGCC TACAACTTTC  
25 2881 TACACCTGTG TCGCTCAGTG TACAGTTACC TATATTTAAT ATTACAATTA TATCATTATT  
2941 AACGAATGAT AAGATTTTAT TAACATTAAT TTCTCTGTCT GAACGTATCA CTGTAAATAT  
3001 TACTAAATGT TTCCTAATTA CATTATTCAT ACATATATTA TCATCCCTTG AGCTATAGTT  
3061 GCAAAGTATT CCAAAACCAC AATGAAAATA AAATTTCAT TTACTTCACG ATCACCAAAT  
3121 TGTGAAAACC TGGTTGTTCT GAATTCATTT AACAATTAGT TTTTACTTTG AATCCATGGC  
30 3181 TCAAGGGACA TCCTAAGGAT ATTCAATGAA ATCTATTTAG AATCTCGTGT ATGTATCATG  
3241 ACACCTTCAA ATAAATATC ACTAATGCTG TGTTCGGCTA TTAGATACAA TAAGTCGTAC  
3301 ATATTACGT AAGCACATTC GTTTTTATTA TGCGCGGAG AGAACGCATC TGTTCCTATA  
3361 ACGAAAGGT GCCATTATC GGCTATATCA TCTTGCTTGG TCTGTATAAA AATAAGAGTC  
3421 AAAGACTCGG GGGAAACCCC TATATGTATA CTATCATAAC CGTTATCCTT ATTTTGACAA  
35 3481 AGCTCTGGGA AACGAAATAG CATTTTGTTC CAATTACACA ATTCTTGCTC ATTTTCTCT

5        3541 TCCGCCTTTT ATTTGAATTT AGGTGTTGCC CACTGTGCGC AATACTCTAA TGGCTTAGAA  
       3601 TTATCCTTAA TATATATTCT CGGGCTGTGA CGAGGTGTAG CATCTGCATT ATTATATTAA  
       3661 TGTCAATTTCG TTIGCCATTC GTTGTATGTA AGGAAATATT AGCCTATGTC CAACGCTCAA  
       3721 AATCTCATAG ACGTATTAGG CACACATAAG TGTACCTTTT CGTATGTATG TAAATTATTG  
       3781 GAGACTCAAT GTCTTAGTTG GTGCTATATA TACTACGATC CGAGGAGAAT GTACCCAGTA  
 10       3841 GTTTACTCAT ACATAACGCC ACTGATATCT TGTGGAGGAA ATATTATCTG CGAGACAAGT  
       3901 AGACATTAGT TAAGTTTACA TATTACAAT AAATGTTTCC ATTATTAGGA TATAACATAT  
       3961 GAATGTGTTA CTGTTGAAAG CAGCTTCTCA AGGTACCACC AGTAATTCGG AGATACTTGT  
       4021 AGGATTTGCA TTCGATAAAC AACTTATACT AAAACGAAGA TTTGACTGAA TCTAAACCGC  
       4081 AAATACTGTG GTCAAAATTA TTAAACACTT TCAATACATG TTGTACGCAT GTTCTGTAA  
 15       4141 TTTACATTTT AATTGTAAAG TCAATTAAAT CACTGTATAA TAATACATTT TCAACATATC  
       4201 TCTCACTGTT AAGATTTTCG TTGGTCCAAC GACAGAATCA AATCGCAACG TAATGATGAT  
       4261 CCGGGCAAAA CTAACAACCTA GATAGATCTC TTAAATGATT ACGTTGAAGT GGAAGAGGTG  
       4321 ATGTATGAAG GAAGGTAGGA TTAAGTAACA CTGTATAATA TATIGACCAT AATTACGATT  
       4381 TTAGAAGTCA TAATGGACGG TTTACCTCTT AAGATTATAC AGTAAAGGTA GATAGTTTCA  
 20       4441 TTCGTAAGCT ATGTTGTACT CGATTGGTAT GACATAACTA ATGACTGAGC TTTGTCATCT  
       4501 ACTACAACCC GAGGGCGAAT ACCTCCTTCT TCTACCATTC CCATTTAATT ATAAGAAAC  
       4561 ATTGTAAGAA ATGATTTAAT AAAATATCCC AAATATCTTA AACAAAAAA AAAAAAAAAA  
       4621 A

Sequencing indicated that HEGE2 encoded a full length *Heliothis virescens* glutamate-  
 25 gated chloride channel clone directionally cloned into the EcoRI and XhoI sites of phagemid  
 pBluescript SK (+/-). The coding sequence starts at 144 bp and ends at 1484 bp, and encodes a  
 polypeptide of 444 amino acids having the predicted sequence (SEQ ID NO: 14):  
 MDIPRPSCALVLLFVTHLSECMNGGKINFREKEKQILDQILGPGRYDARIRPSGINGTDGP  
 AVVSVNIFVRSISKIDDVTMEYSVQLTFREQWLDERLKFNNLGGRLKYLTLTEANRVWM  
 30 PDLFFSNEKEGHFHNIIMPVYIRIFPNGNVLYSIRISLTLSCPMNLKLYPLDKQTCSLRMA



5 SYGWTDDDLVFLWKEGDPVQVVKNLHLPRFTLEKFLTDYCNSKTNTGEYSCLKVDLLF  
KREFSYYLIQIYIPCCMLVIVSWVSFWLDQGAVPARVLLGVTTLLTMATQSSGINASLPPV  
SYTKAIDVWTGVCLTFVFGALLESRFVNYASRSDMHRENMKKARREMEAASMDAASD  
LLDTSNTTFAMKPLVRGGVVESKMRQCEIHITPPRKNCRLWMSKFPTRSKRIDVISRIT  
FPLVFALFNLAYWSTYLFREDEEEK

10 BLAST search

BLAST search (<http://www.ncbi.nlm.gov>) and amino acid sequence comparison were used to identify glutamate-gated chloride channel-like fragments from the PCR products amplified with degenerate primers, the PCR products obtained from RACE and clones screened from embryo cDNA library. BLAST was also used to determine the orientation and the position of the amplified products compared to the entire cDNA sequence.

**EXAMPLE 3**

Expression of Nucleic Acid Encoding Lepidopteran Glutamate-gated Chloride Channel in *Xenopus* Oocytes:

Messenger RNA was produced from the cDNA template of HEGE2 by in vitro transcription with the Ambion mMESSAGE mMACHINE IN VITRO TRANSCRIPTION KIT (Ambion, Inc.). The mRNA was injected into oocytes by the following procedure.

Frogs were anesthetized in a 2 gram/liter solution of 3-amino benzoic acid ethyl ester for thirty minutes, after which oocytes were surgically removed from the abdominal cavity. Follicles

5 were digested by collagenase treatment under sterile conditions by standard methods. Oocytes were injected with 50 nl of 1  $\mu\text{g}/\mu\text{l}$  mRNA by glass electrodes.

After a 24 hr incubation, two-electrode voltage clamp recordings were made. Recordings were made using a Dagan, TEV200 voltage clamp interfaced with a MacLab4 data acquisition system running the MacLab Chart data acquisition/analysis software. Oocytes were positioned  
10 under a dissection scope under constant perfusion with frog saline (96 mM of NaCl, 2 mM of KCl, 1 mM of  $\text{MgCl}_2$ , 1.8 mM of  $\text{CaCl}_2$  and 10 mM of HEPES, pH 7.5) using a Razel syringe perfusion pump. Model A99-FY at 93.9 cc/hr. Glass electrodes (A-M Systems, Inc. 1.5 mm x 0.86 mm) were filled with 3 M KCl and resistance (a function of the diameter of the channel opening) was measured to be between 0.7 and 1.5 mega ohms. Both electrodes were inserted  
15 into the oocyte at opposite sides, the resting potential was recorded and the voltage clamp turned on. Oocytes were held at a resting potential at  $-80$  mV. Control responses of glutamate were obtained by stopping the perfusion of saline and perfusing with a known concentration of glutamate in frog saline. The average of several glutamate applications was taken as the maximal chloride current for that particular glutamate dose. The effect of 100 micromolar glutamate on  
20 the lepidopteran glutamate-gated chloride channel expressed in *Xenopus* oocytes is depicted in FIG. 1. Glutamate application is indicated by the arrow marked "on". The data in FIG. 1 indicate that 100uM glutamate activate a membrane current in *Xenopus* oocytes injected with 50 ng of HEGE2 mRNA. This example demonstrates that the expression of mRNA corresponding to the cDNA in HEGE2 results in a functional glutamate gated chloride channel in oocytes. One  
25 micromolar of Ivermectin phosphate also slowly and irreversibly activated current in oocytes.